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FUNGICIDAL AND BACTERICIDAL ACTIVITY OF THE ALKYL-SUBSTITUTED GUANIDINE-CONTAINING OLIGOMERS

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Biocides are widely used in medicine and various industries to protect against a number of harmful microorganisms. Organic quaternary ammonium and guanidine-containing compounds, the biological action of which is based on membrane-toxic properties, are used as bactericidal preparations. ***The aim*** *of this work was to study the bactericidal and fungicidal activities of the synthesized oligomeric alkyl-substituted guanidinium bromides with different radicals -C_3H_7, -C_7H_{15}, -$C_{10}H_{21}$, against different isolates of heterotrophic bacteria and microscopic fungi.* ***Methods.*** *The synthesis of alkyl-substituted guanidinium-containing oligomers was performed in two stages. In the first stage, alkyl-substituted guanidine was obtained by the reaction of guanidine, previously converted by alkali from the salt form to the base form by the base and alkyl bromides (Alk = -C_3H_7 (propyl), -C_7H_{15} (heptyl), -$C_{10}H_{21}$ (decyl)) in methanol at a temperature of 50° C and a molar ratio of 1:1. The second carried out the reaction between aromatic oligoepoxide DER-331 and alkyl-substituted guanidine in methanol at a temperature of 50° C for 2–3 hours and a molar ratio of 1:2. Bacteria were grown on meat-peptone agar for 48 hours at a temperature of 28±2° C. Test cultures of micromycetes were cultured on agar beer wort (6° B), incubated for 14 days in a thermostat at a temperature of 28±2° C. Antimicrobial activity of newly synthesized alkyl-substituted guanidinium-containing oligomers was determined by standard disco-diffusion method (method of disks on agar) and fungicidal activity was determined by the method of holes in agar.* ***Results.*** *Oligomeric alkyl-substituted guanidinium bromides with different radicals composed -C_3H_7, -C_7H_{15}, -$C_{10}H_{21}$- synthesized by the reaction of guanidine alkyl bromides with aromatic oligoepoxydes. It was found that alkyl-substituted guanidinium-containing oligomers at a concentration of 1–3 % inhibited the growth of Escherichia coli 475, Pseudomonas aeruginosa 465, Klebsiella pneumonia 479, Pseudomonas pseudoalcaligenes 109, Staphylococcus aureus 451, E. faecalis 422, Rhodococcus erythropolis 102, Bacillus subtilis 138 and most of the studied micromycetes – Aureobasidium pullulans F-41430, Paecilomyces variotii F-41432, Penicillium funiculosum F-41435, Penicillium ochrochloron F-41431, Scopulariopsis brevicaulis F-41434, Trichoderma viride F-41437, Candida albicans F-41441, Aspergillus flavus F-41442, Aspergillus niger F-41448, Penicillium sp. F-41447.* ***Conclusions****. Antimicrobial and fungicidal properties significantly depend on the length of the alkyl radical, with increasing of its length the diameter of the zone of bacterial and micromycetes growth retardation increases.*

Keywords: alkyl-substituted guanidinium-containing oligomers, heterotrophic bacteria, micromycetes, bactericidal and fungicidal activity.

Biocides are widely used in medicine and various industries as a reliable means of protection against a number of microorganisms. The biocides include chemicals: alcohols, phenols, oxidants (hydrogen peroxide, potassium permanganate, iodine, iodoform, chlorine, chloramine), salts of heavy metals (copper, mercury, silver). According to the action mechanism, the substances can coagulate proteins, oxidate sulfhydryl groups in protein structures [1]. As bactericidal preparations,

organic quaternary ammonium compounds are also used, which are well established as an effective means of extinguishing sulfate-reducing bacteria on oil fields [2–4]. The biological effect of quaternary ammonium salts is to break the structure of cell membranes and to cause denaturation of cell proteins, as well as to reduce the activity of key enzymes [1].

Guanidine derivatives are widely used as antiseptics, insecticides, drugs and preservatives [5]. Guandinium chloride belongs to the second class of hazard [5]. To date, a number of drugs based on guanidine have been developed, such as biguanidines, sulfaguanidine and chlorhexidine. Among the polymer derivatives of guanidine, polyhexamethylene guanidine chloride (PHMGC) has been most widely used in practice [6–8]. The mechanism of biocidal action of polyguanidines is similar to quaternary ammonium compounds and is of membrane-toxic nature: guanidine polycations are adsorbed on the negatively charged surface of bacterial cells; diffuse through the cell wall; bind to acidic phospholipids, proteins of the cytoplasm membrane, which causes its breakage. As a result, the microbial cell dies. The increase in the biocidal activity of PHMGC as compared to the low-molecular biocides is due to the cooperative interaction of the adjacent guandine polycation group links with the microbial cell. The increase in the activity of polyguanidines as compared to quaternary ammonium salts (QAS) is also due to the peculiarities of the guandine group structure: unlike QAS cation, in which a large positive charge is localized on a single nitrogen atom, guanidin cation has a positive charge distributed between three nitrogen atoms, and additionally delocalized according to the σ-links system. It is well known that guanidinium polymers are less toxic than guanidine and belong to the third class of hazard [9–13].

Speaking of bactericidal properties, a lot of attention is drawn by the class of guanidinium, oligomers which have not been properly researched yet. The possibilities of changing within the wide limits of the structure and physical and chemical properties of oligomers, considering the unique role of terminal groups, can open their new functional capabilities as bactericidal substances. It is possible to assume that by analogy with ammonium derivatives of organic compounds, guanidinium oligomers can exhibit bactericidal activity against both gram-positive and gram-negative bacteria, as well as fungicidal properties against microscopic fungi.

We have previously studied fungicidal activity of the guanidinium-containing oligoether (GO) with terminal guanidinium fragments based on aromatic oligoepoxide and guanidinium chloride in relation to the microscopic fungi isolates, which caused damage to rubber materials. It has been found that guanidinium-containing oligoether at a concentration of 3 % inhibited the growth of most micromycetes that have been studied [14–17]. It is well known that the addition of alkyl radicals of different lengths to the chain leads to an increase in bactericidal and fungicidal effects of the resulting compounds. In order to enhance these properties, as well as to simplify the synthesis process and to reduce its cost, it is deemed beneficial to obtain reactive functional guanidinium-containing oligomers that contain alkyl radicals of different lengths.

The aim of this work was to study bactericidal and fungicidal activities of synthesized oligomeric alkyl-substituted guanidinium bromides with different radicals $–C_3H_7$,- C_7H_{15}, -$C_{10}H_{21}$-, against bacteria and micromycetes.

Materials and methods

Study subjects were strains of gram-positive *Staphylococcus aureus* 451, *Enterococcus faecalis* 422, and gram-negative bacteria *Escherichia coli* 475, *Pseudomonas aeruginosa* 465, and *Klebsiella pneumonia* 479, isolated from pathogenic material (urogenital system) of patients and stored in the Bacteriological study laboratory of the Urology Institute of the National Academy of Medical Sciences of Ukraine. Strains of bacteria *Pseudomonas pseudoalcaligenes* 109, *Rhodococcus erythropolis* 102, *Bacillus subtilis* 138 have been previously isolated from the damaged protective coatings of gas pipelines, identified by us, and are now stored in the collection of the Department of General and Soil Microbiology of Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine [18].

Microscopic fungi, isolated from Kyiv premises which experienced mycological damage, *Aureobasidium pullulans* F-41430, *Paecilomyces variotii* F-41432, *Penicillium funiculosum* F-41435, *Penicillium ochrochloron* F-41431, *Scopulariopsis brevicaulis* F-41434, *Trichoderma viride* F-41437, *Candida albicans* F-41441, *Aspergillus flavus* F-41442, *Aspergillus niger* F-41448, *Penicillium* sp. F-41447 are stored in the collection of the Department of Micromycete Physiology and Systematization of Zabolotny Institute of Microbiology and Virology of the

National Academy of Sciences of Ukraine [19].

Study materials. The diane epoxy oligomer DER-331 (DOW Chemical Company, Germany), MM 365 g/mol, weight fraction of epoxy groups 23.5 %, hydroxyl groups 0.6 %, was dried by heating under vacuum for 2–6 hours at 80–90 °C and residual pressure of 2 mm Hg. Guanidine hydrochloride (GH) ("Aldrich," Germany) was used without further purification. Alkyl bromides, namely propyl- ($-C_3H_7$), heptyl- ($-C_7H_{15}$), and decyl- ($-C_{10}H_{21}$) bromides ("Aldrich," Germany) were used without further purification. Methanol was purified by means of distillation.

The synthesis of alkyl-substituted guanidinium containing oligomers was carried out in two steps. In the first step, alkyl-substituted guanidine was obtained by reacting guanidine previously converted by means of alkali from salt form into basic form, and alkyl bromides (Alk = $-C_3H_7$ (propyl), = $-C_7H_{15}$ (heptyl), $-C_{10}H_{21}$ (decyl)) in methanol at 50° C and a molar ratio of components 1:1. In the second step, aromatic olygoepoxide DER-331 was reacted with alkyl-substituted guanidine in methanol for 2–3 hours at 50° C and a molar ratio of components 1:2. The reaction completion was controlled by infrared spectroscopy, by disappearance of epoxide absorption bands at 920 cm^{-1}. The resulting product was re-deposited from methanol to diethyl ether. To remove solvent residues, the product was kept under vacuum at 60° C for 5 hours. The product yield was 95 %.

Cultivation of microorganisms. Bacteria were grown on Meat-peptone agar for 48 hours at 28±2° C.

The test cultures of micromycetes were cultivated on agar wort (6° B) for 14 days in the thermostat at 28±2° C.

The antimicrobial and fungicidal activity. Antimicrobial activity of newly synthesized alkyl-substituted guanidinium-containing oligomers was determined by standard disco-diffusion method (method of disks on agar) [6] and fungicidal activity was determined by the method of holes in agar. 1 and 3 % oligomer solutions were used: 0.2 ml of each was applied to the standard paper discs (6 mm in diameter), and placed on the surface of Meat-peptone agar inoculated with the corresponding test culture of bacteria. Incubation was carried out within 18 hours at 28±2° C. Antimicrobial activity was expressed according to the diameters (mm) of the microorganism growth inhibition zones.

The fungicidal activity was determined by the method of holes in agar. In order to determine the fungicidal activity, a suspension of each fungi species was prepared in sterile distilled water at a concentration of $1x10^6$ CFU. 1 ml of suspension was added to a Petri dish, 20 ml of molten Czapek-Dox medium was poured over it, and it was thoroughly mixed. Then in the middle of the dish, wells (8 mm in diameter) were made by a sterile drill, and 0.2 ml of the test substances was added. Incubation was carried out at 28± ±2° C, accounting of the results was made on the 7^{th} day of the experiment. Fungicidal activity was expressed in mm according to the microorganism growth inhibition zone diameters. The study was carried out in triplicate; the obtained results were processed mathematically on a personal computer.

Fourier-transform infrared spectroscopy. Infrared spectral data of oligomers with Fourier transformation were collected on "TENSOR 37" spectrophotometer in the spectral region 6000–400 cm^{-1} in KBr tablets.

^{1}H NMR spectral data were collected on "Varian VXR-400 MHz" (USA) device in $CDCI_3$ system.

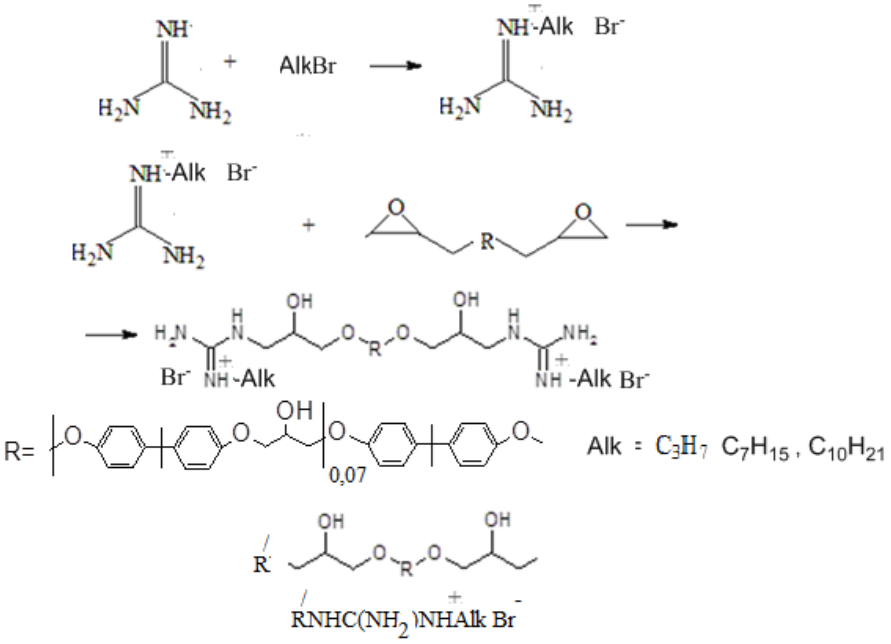
Statistical processing of the results. The experiments were carried out in triplicate, and the results were expressed in terms of the mean square deviation M ± n. Data processing was performed using Exel 2016 (MS Office) and Origin 8.5 (MS Office).

Results. For our experiments the new alkyl-substituted guanidinium oligomers were synthesized. Stages of obtaining the new alkyl-substituted guanidinium oligomers are presented on Fig. 1.

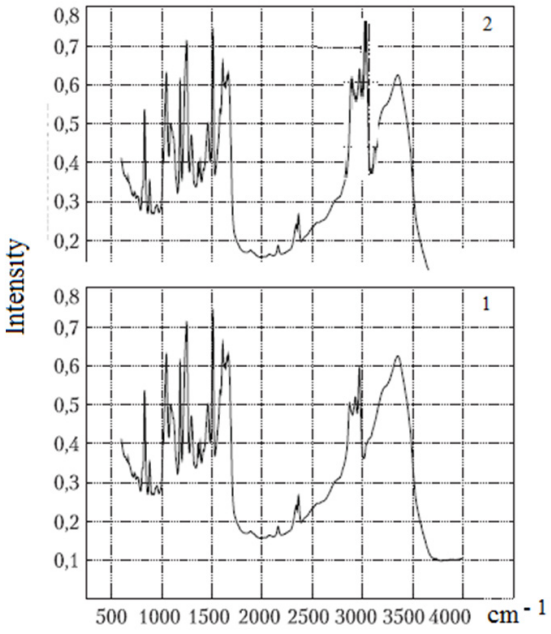
The obtained oligomers structure was confirmed by IR spectroscopy. In the obtained product IR spectrum within the range of 3200–3550 $см^{-1}$ there are bands of valence vibration absorption of -OH and -NH groups. The presence of -CH, $-CH_2$ and $-CH_3$ groups is confirmed, respectively, according to the absorption bands 2869 cm^{-1}, 2926 cm^{-1}, 2964 cm^{-1}, which correspond to the valence vibration of C-H bonds; the bands of deformation vibration of these bonds are within 1460 cm^{-1}. With the increase of the alkyl radical length the intensity of their valence vibration band was also increasing. The band of valence vibration absorption of -C=N guanidine fragments was observed at 1640 $см^{-1}$, which overlapped the band of deformation vibration of -NH groups. In the range of 1450–1650 $см^{-1}$ there are absorption bands of -C=C-bonds of benzene ring. Absorption bands in the frequency range of 1100–1300 $см^{-1}$ indicated the fluctuations of C-O-C bonds of etheric groups. In comparison

with the initial products, the absorption bands of epoxy groups disappear within the limits of 920 см$^{-1}$ (Fig. 2).

The structure of the obtained oligomers has been confirmed by ^{1}H NMR spectrometry. In ^{1}H NMR ($CDCI_3$) spectrum of alkyl-substituted guanidinium containing oligomers there are proton signals at 1.72 ppm (t, 3H, -CH_3(a)), 2.73 ppm -NH (NH-CH_2(c)), 2.58 ppm -CH_2 (CH_2CHOH(b)), 3.58 ppm -OH (CH-OH(d)), 3.96 ppm -CH (CH-OH(e)), 6.8 ppm and 7.2 ppm -CH (f) of benzene ring, 8.4 ppm and 8.6 ppm -NH (NH_2 groups (f)).



F i g. 1. Stages of obtaining the new alkyl-substituted guanidinium oligomers



F i g. 2. Infrared spectra of alkyl-substituted guanidinium oligomers: 1 – Alk=C_3H_7, 2 – Alk=$C_{10}H_{21}$

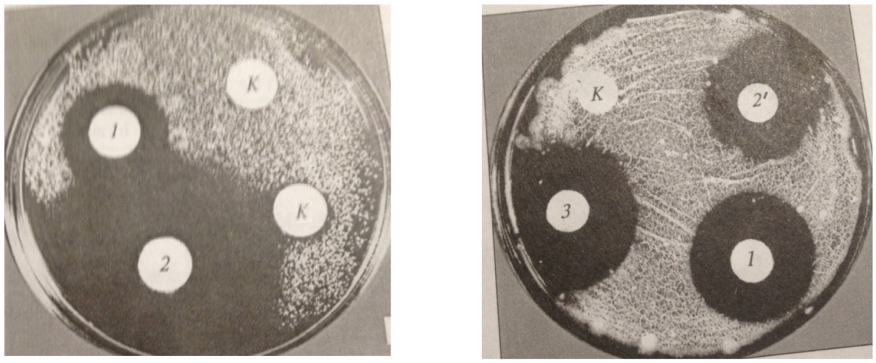
Thus, we obtained the confirmation that the new alkyl-substituted guanidinium containing oligomers have a general formula R$^/$NHC(NH_2)NH^+AlkBr$^-$ with different alkyl radicals in their composition Alk=C_3H_7, C_7H_{15}, $C_{10}H_{21}$.

These are viscous light yellow liquids which are soluble in water, ethanol, methanol, methylethylketone, dimethylformamide, dimethylsulfoxide, dimethylacetamide, and insoluble in diethyl ether, hexane, and acetone. The synthesized alkyl-

substituted guanidinium containing oligomers are multifunctional compounds with hydrophobic aromatic component, alkyl substitutes, they contain hydroxyl groups and guanidinium fragments.

The bactericidal activity of the newly synthetized alkyl-substituted guanidinium containing oligomers was tested. Some test cultures of bacteria were selected, which differed by the cell wall structure – gram-positive and gram-negative bacteria (Fig. 3, Table 1).

According to the microbiological study results, in most cases the obtained compounds had antimicrobial activity against the studied test cultures of bacteria at 1–3 % concentrations. The bacterial growth inhibition zone diameter was in the range of 8–40 mm. As it can be seen from the data, antimicrobial properties of the newly synthetized compounds significantly increased with the increase of the alkyl radical length, which is confirmed by higher bacterial growth inhibition zone diameter. For example, after the influence of 3 % solution of the test compound Alk = C_3H_7, the diameter of the growth inhibition zone of gram-negative *E. coli* strain was 6 ± 0.08 mm, with the growth of alkyl radical Alk = $C_{10}H_{21}$ in the guanidine oligomeric compound, the growth inhibition zone increased up to 18 ± 0.28 mm. The highest values of the growth inhibition zone (from 12 ± 0.25 up to 18 ± 0.28 mm) of gram-negative bacteria *(E. coli* 475, *K. pneumonia* 479, *P. aeruginosa* 465) have been noted with the effect of oligomer with the biggest radical length Alk = $C_{10}H_{21}$. Gram-positive soil bacteria *R. erythropolis* 102 (30–40 mm) and *B. subtilis* 138 (20–30 mm) were found to be sensitive to oligoetherguanidiniumdecyl bromide (Alk=C_7H_{15} and Alk=$C_{10}H_{21}$). The tested oligomers did not exhibit any bactericidal effect against *P. pseudoalcaligenes* 109.



a **b**

F i g. 3. Growth inhibition zones of *R. erythropolis* 102 (a) and *B. subtilis* 138 (b) affected by alkyl-substituted guanidine containing oligomers: К – control (distilled water), 1, 2′ – 1 % solution (Alk= $C_{10}H_{21}Br$), 2, 3 – 3 % solution (Alk= $C_{10}H_{21}Br$)

Table 1

Bactericidal activity of alkyl-substituted guanidinium containing oligomers

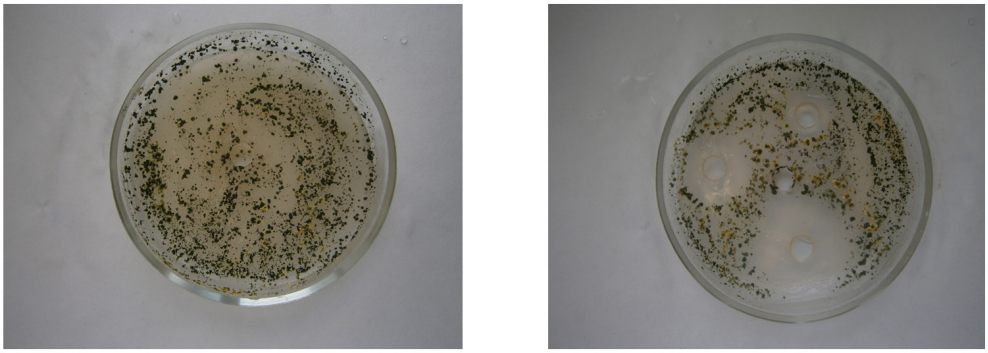
Chemical group	Growth inhibition zone diameter, mm					
	Alk=C_3H_7		Alk=C_7H_{15}		Alk=$C_{10}H_{21}$	
	Oligomer concentration, %					
Bacteria strains	1	3	1	3	1	3
Gram-negative bacteria						
E. coli 475	13±0.26	6±0.08	16±0.35	16±0.35	13±0.26	18±0.28
P. aeruginosa 465	9±0.10	10±0.15	11±0.10	11±0.23	11±0.10	12±0.25
K. pneumonia 479	8±0.10	0	13±0.26	13±0.26	12±0.25	14±0.30
P. pseudoalcaligenes 109	–	–	0	0	0	0
Gram-positive bacteria						
S. aureus 451	16±0.35	10±0.20	17±0.36	12±0.25	15±0.34	20±0.30
E. faecalis 422	10±0.15	8±0.10	10±0.15	19±0.35	12±0.23	12±0.25
R. erythropolis 102	–	–	30±0.44	30±0.46	35±0.45	40±0.50
B. subtilis 138	–	–	20±0.30	25±0.32	25±0.32	30±0.43

Legend: «– » not tested, 0 – no oligomer effect.

Thus, the highest bactericidal activity against gram-positive bacteria was exhibited by the guanidinium containing oligomer with alkyl substitute Alk-$C_{10}H_{21}$; the bacteria growth inhibition zone diameter was 20–40 mm.

The study data of the microscopic fungi test cultures fungicide activity are shown on Fig. 4 and in Table 2.

According to the obtained data alkyl substituted (Alk=C_7H_{15} and $C_{10}H_{21}$) guanidinium containing oligomers with 1 % concentration exhibit fungicidal activity against almost all studied isolates. The diameter of the growth inhibition zone of such strains as *A. pullulans* F-41430, *P. variotii* F-41432, *P. funiculosum* F-41435, *P. ochrochloron* F-41431, *S. brevicaulis* F-41434, *T. viride* F-41437, *C. albicans* F-41441, *A. flavus* F-41442, *A. niger* F-41448, *Penicillium* sp. F-41447 affected by the obtained oligomers was found to be 7–25 mm depending of the fungi type. The biggest diameter of the growth inhibition zone was observed for fungi *A. pullulans* F-41430, *P. variotii* F-41432 and *T. viride* F-41437 – 19–25 mm, the smallest one – for fungi *A. flavus* F-41442, *Penicillium* spp F-41447, *A. niger* F-41448 – 7–8 mm for oligomer with decyl radical (Alk=$C_{10}H_{21}$). The oligomer with Alk=C_3H_7 did not exhibit any fungicidal activity against any of the studied test cultures of micromycetes. Thus, the oligomer with alkyl substitute $C_{10}H_{21}$ exhibits the highest fungicidal activity.



a **b**

F i g. 4. Growth inhibition zones of *T. viride* F-41437 affected by alkyl-substituted guanidine-containing oligomer with decyl bromide radical ($C_{10}H_{21}$): a – control without the addition of compounds, b – impact on *T. viride* F-41437 (study in three repetitions)

Table 2
Fungicidal activity of alkyl-substituted guanidinium-containing oligomers (concentration 1 % in water solution)

Chemical group / Fungi type	Fungi growth inhibition zone diameter, mm		
	Alk=$C_{10}H_{21}$	Alk=C_7H_{15}	Alk=C_3H_7
A. pullulans F-41430	25.0±1.60	25.0±1.24	0
P. variotii F-41432	23.0±1.16	19.0±0.80	0
T. viride F-41437	19.3±1.85	20.7±1.16	0
S. brevicaulis F-41434	18.3±0.80	15.8±0.65	0
C. albicans F-41441	16.0±0.65	12.0 ±0.33	0
P. ochrochloron F-41431	14.2±1.05	10.2±0.2	0
P. funiculosum F-41435	10.5±0.33	0	0
P. spp. F-41447	8.0±0.20	0	0
A. flavus F-41442	7.0±0.11	0	0
A. niger F-41448	7.0±0.20	0	0

Legend: 0 – no growth inhibition.

Discussion. In our study we used the strains of bacteria from the collection of Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, and Institute of Urology of the National Academy of Medical Sciences of Ukraine. The bacteria we have researched, *P. pseudoalcaligenes* 109, *R. erythopolis* 102 and *B. subtilis* 138, form extracellular

polysaccharides and lipopolysaccharides, thanks to which bacteria adhere to the protective materials surface, which ensures their rapid reproduction and contributes to the survival of the population under extreme conditions. These exopolysaccharides differed by monosaccharide composition and fatty acid composition. Analysis of the fatty acid composition of the exopolymeric complex of bacteria has revealed the presence of saturated, unsaturated fatty acids and oxy-acids with carbon chain length 11–18 carbon atoms [20].

Previously it has been demonstrated that corrosion inhibitors with biocidal action, namely the salt of cyclohexylamine and synthetic fatty acids (C_6 H_{11} NH_2 HOOCR, where R=C_9….C_{11}) inhibited the processes of denitrification and sulfate reduction as a result of which corrosive metabolites (NO^-_2, NH_3, H_2S) are formed. Slowing down of the mentioned processes by these inhibitors reduced the aggressiveness of the medium [18]. The synthetized guanidine-containing polyethyleneoxide hydrogel which demonstrated antimicrobial activity against hydrocarbon oxydative bacteria *R. erythropolis* 102 and *B. subtilis* 138 –destructors of protective materials, promoted the decrease of catalase and lipase activities of these microorganisms [21].

It can be assumed that the negative effect of the obtained oligomers against microorganisms is based on one of the following mechanisms: a) biocide penetrates the cell, binds to some of its components (proteins, lipids, carbohydrates), as a result of which metabolism is disrupted, which leads to death; b) biocide does not penetrate the cell, but it blocks the activity of lipid and protein substances (in particular enzymes or their active centers) on the cell surface, which causes negative lethal changes in membrane functions [2].

The fungicidal effect of the studied oligomers is likely due to the presence of a diphenylpropane group in their composition. It is well known that in the organic substance molecules under the influence of various atoms or atomic groups which are a part of them, there takes place redistribution of electronic density of chemical bonds (positive or negative induction effect). The presence of a diphenylpropane group in a GO molecule causes negative induction effect – the substituent reduces the electron density on the carbon atom to which it is bound. The substituent thus acquires partial negative charge (δ-) and the carbon atom acquires partial positive charge (δ+) [11]. According to the literature, the value (δ+) may be one of the factors that enhance the interaction of fungicidal substances with the cell wall of fungi [13].

Macromolecules of guanidine polymers are known to adsorb on the negatively charged cell surface, thus blocking the processes of breathing and nutrition (transporting of metabolites through the cell wall and cytoplasmic membrane). They diffuse through the cell wall, causing irreversible damages within the cell and inactivation of a number of enzymes [9]. The low toxicity of such compounds for humans and warm-blooded animals is worth noting [10]. Bactericidal and fungicidal activity can be considered to occur through a similar mechanism in the compounds that have been studied by us. In comparison with the guanidinium containing oligomer with terminal guanidine fragments that we have previously studied [14–16], the growth inhibition zones of micromycetes *A. flavus* F-41442*, Penicillium* spp F-41447*, A. niger* F-41448, were 21, 43 and 73 mm, respectively, which considerably exceeds the indicators for alkyl substituted guanidinium containing oligomers that we have researched.

If we compare the structure of the synthetized alkyl substituted guanidinium containing oligomers to the guanidinium containing oligomer with terminal guanidine fragments that we studied before, they differ in structure by the presence of alkyl radicals and a considerably smaller concentration of diphenylpropane groups and guanidine fragments. The obtained data demonstrate selectivity of the fungicide activity against different types of microscopic fungi, which can be related to the differences in their metabolic processes and adaptation mechanisms.

Conclusions. Guanidinium containing oligomers with alkyl substitutes C_7H_{15}, $C_{10}H_{21}$ exhibit antimicrobial activity against the studied test cultures of bacteria at 1–3 % concentration, and fungicidal activity against microscopic fungi at 1 % concentration. With the increase in the length of the alkyl radical of guanidinium containing oligomers their bactericidal and fungicidal properties increase significantly. Alkyl substituted oligomers based on guanidine are beneficial for usage as desinfectants for treatment of premises and as additives in polymeric compositions for protecting them from biodegradation.

ФУНГІЦИДНА ТА БАКТЕРИЦИДНА АКТИВНОСТІ АЛКІЛЗАМІСНИХ ГУАНІДИНІЙВМІСНИХ ОЛІГОМЕРІВ

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Резюме

Біоциди широко використовуються в медицині та різних галузях промисловості як надійний спосіб захисту від ряду мікроорганізмів. Як бактерицидні препарати використовують органічні четвертинні амонієві сполуки, біологічна дія яких полягає у порушенні структури клітинних мембран і спричиненні денатурації клітинних білків, зниженні активності ключових ферментів. Похідні гуанідину широко застосовують як антисептики, інсектициди, лікарські засоби та консерванти. Серед полімерних похідних гуанідину найбільшого практичного застосування отримав полігексаметиленгуанідинійхлорид (ПГМГХ). Механізм біоцидної дії полігуанідинів подібний четвертинним амонієвим сполукам і носить мембрано-токсичний характер. Відомо, що гуанідинієві полімери мають меншу токсичність у порівнянні з гуанідином і відносяться до третього класу небезпеки. За бактерицидними властивостями значну увагу привертає практично не досліджений клас гуанідинієвих олігомерів. З метою посилення антимікробних властивостей та спрощення і здешевлення процесу синтезу видається перспективним отримання реакційноздатних функціональних гуанідинійвмісних олігомерів з алкільними радикалами різної довжини в своєму складі. **Метою** даної роботи є дослідження бактерицидної і фунгіцидної активностей синтезованих олігомерних алкілзамісних гуанідинійбромідів з різними радикалами -C_3H_7,- C_7H_{15}, -$C_{10}H_{21}$- по відношенню до бактерій та мікроміцетів. **Методи.** Синтез алкілзамісних гуанідинійвмісних олігомерів проводили двостадійно. На першій стадії отримували алкілзамісний гуанідин за реакцією гуанідину та алкілбромідів (Alk= -C_3H_7 (пропіл), -C_7H_{15} (гептил), -$C_{10}H_{21}$ (децил)) у метанолі та мольному співвідношенні компонентів 1:1. На другій – проводили реакцію між ароматичним олігоепоксидом DER-331 та алкілзамісним гуанідином в метанолі при мольному співвідношенні компонентів 1:2. *Культивування мікроорганізмів*. Бактерії вирощували на м'ясо-пептонному агарі протягом 48 годин за температури 28±2° С. Тест-культури мікроміцетів культивували на агаризованому пивному суслі (6° Б), інкубували 14 діб у термостаті за температури 28±2^0 С. Антимікробну активність новосинтезованих алкілзамісних гуанідинійвмісних олігомерів визначали стандартним диско-дифузійним методом (метод дисків на агарі) та фунгіцидну активність –методом лунок в агарі. **Результати.** Олігомерні алкілзамісні гуанідинійброміди з різними радикалами у своєму складі -C_3H_7,- C_7H_{15}, -$C_{10}H_{21}$- синтезовано за реакцією гуанідину та алкілброміду з подальшою взаємодією з ароматичним олігоепоксидом. Показано бактерицидну та фунгіцидну активності алкілзамісних гуанідинійвмісних олігомерів по відношенню до різних гетеротрофних бактерій та мікроскопічних грибів. Встановлено, що алкілзамісні гуанідинійвмісні олігомери за концентраціями 1–3 % інгібували ріст грамнегативних (*Escherichia coli* 475, *Pseudomonas aeruginosa* 465, *Klebsiella pneumonia* 479) і грампозитивних (*Staphylococcus aureus* 451, *Enterococcus faecalis* 422, *Rhodococcus erythropolis* 102, *Bacillus subtilis* 138) бактерій. Найвищі значення зони пригнічення росту (від 12±0,25 до 18±0,28 мм**)** грамнегативних бактерій (*E. coli* 475, *K. pneumonia* 479, *P. aeruginosa* 465) відмічено за дії олігомеру з найбільшою довжиною радикалу Alk=$C_{10}H_{21.}$ Чутливими до олігоетергуанідинійдецилброміду (Alk=C_7H_{15} і Alk=$C_{10}H_{21}$) виявились грампозитивні ґрунтові бактерії *Rhodococcus erythropolis* 102 (30–40мм) і *Bacillus subtilis* 138 (20–30мм). Гуанідинійброміди з довжиною алкільного радикалу від Alk=C_7H_{15} до Alk=$C_{10}H_{21}$ у концентрації 1 % чинили бактерицидну дію на мікроміцети –*Aureobasidium pullulans* F-41430, *Paecilomyces variotii* F-41432, *Trichoderma viride* F-41437, *Scopulariopsis brevicaulis* F-41434, *Candida albicans* F-41441, *Penicillium ochrochloron* F-41431. **Висновки.** Зі збільшенням довжини алкільного радикалу гуанідинійвмісних олігомерів суттєво підвищуються їх бактерицидні та фунгіцидні властивості. Алкілзамісні олігомери на осно-

ві гуанідину є перспективними до використання як дезінфектанти для обробки приміщень та як добавки в полімерні композиції для захисту їх від біопошкоджень.

Ключові слова: алкілзамісні гуанідинійвмісні олігомери, гетеротрофні бактерії, мікроміцети, бактерицидна та фунгіцидна активності.

1. Bocharov BV. [Protection against biodeterioration during the restoration of historical and architectural monuments. Biodegradation in construction]. Moscow: Stroyizdat; 1984:177–83. Russian.
2. Oparin PS, Turneva NA, Sheptunov SI. [The past present and the future of Quaternary ammonium compounds]. Disinfection at the present stage: VS NTS SB RAMS, Irkutsk; 2003:53–61. Russian.
3. Demchenko NS. [Quaternary salts of pyridinium as inhibitors of microbial corrosion of low-carbon steel] JCCT. 2017: 11(3):314–18. Russian.
4. Olkhovik VK, Vasilevsky DA, Matvienko YuV, Petushok VG, Zheldakova RA, Lysak VV. [Synthesis and fungicidal activity of Quaternary ammonium salts based on substituted biphenyls]. Scientific and Practical Conference "Biologically Active Substances: Fundamental and Applied Issues of Preparation and Application": Abstract doc. Kiev; Publisher VS. Martyniuk. 2009:137. Russian.
5. Gembitsky PA. [Guanidine Chemical encyclopedia]. In 5 volumes. (Vol.1) Moscow: Soviet encyclopedia. 1988:617 p. Russian.
6. Vointseva II, Gembitsky PA. [Polyguanidines - disinfectants and multifunctional additives in composite materials]. 2009. Moscow:LKM-press (in Russian). 300 p. Russian.
7. Gembitsky PA, Vointseva II. [Polymeric biocide drug polyhexamethylene guanidine]. Zaporozhye: "Polygraph". 1998:44 p. Russian.
8. Vointseva II. [Polyhexamethylene guanidine hydrochloride for the purification and disinfection as an alternative to oxidizing agents]. Part 1. Water: Chemistry and Ecology. 2011; 7:39–45. Russian.
9. Svetlov DA, Erofeev VT, Morozov EA. [Polyhexamethylene guanidine derivatives biocidal preparations]. Biological damage and bio-corrosion in construction: materials of the Second Internet scientific and technical conference. 2006; 270 –73. Russian.
10. Kim J-Y, Kim HH, Cho KH. Acute cardiovascular toxicity of sterilizers, PHMG, and PGH: severe inflammation in human cells and heart failure in zebrafish. Cardiovasc. Toxicol. 2013; 13:148–160.
11. Jung HN, Zerin T, Podder B, Song HY, Kim YS. Cytotoxicity and gene expression profiling of polyhexamethylene guanidine hydrochloride in human alveolar A549 cells. Toxicol. In Vitro. 2014; 28:682–692.
12. Oulè MK, Azinwi R, Bernier AM, Kablan T, Maupertuis AM, Mauler S, et al. Polyhexamethylene guanidine hydrochloride-based disinfectant: a novel tool to fight meticillin-resistant *Staphylococcus aureus* and nosocomial infections. J Med Microbiol. 2008; 57:1523–8.
13. Stelmakh SA. [Hydrogels based on polyhexamethylene guanidine hydrochloride]. Bulletin of the Buryat State University. Chemistry, physics 2010; B3:80–2. Ukrainian.
14. Patent No. 65079. Wortman MYa, Chuenko AI, Lemeshko VN, Klimenko NS, Shevchenko VV, Zhdanova NM. [The use of a guanidine-containing adduct as a fungicidal substance]. publ. 25.11.2011, Bul. 22. Ukrainian.
15. Declaration of Patent for Utility Model No. 114915. Chuenko AI, Pismennaya YB, Vortman MYa, Lemeshko VN, Shevchenko VV. [A Method of Obtaining a Guanidine-Containing Fungicidal Product]. publ. 27.03.2017, Bul. 6. Ukrainian.
16. Chuenko AI, Wortman MYa, Shevchenko VV. [Fungicidal activity of guanidine derivatives promising for use in the rubber industry]. Microbiol and Biotechnol. 2013; 22(2):97–106. Ukrainian.
17. Dudka IA, Vasser SP, Ellanskaya IA and others. [Methods of experimental mycology]. In: Bilay VI, editor. Kiev: Naukova Dumka, 1982: 550 p. Russian.
18. Andreyuk KI, Kozlova IP, Kopteva ZhP, Pilyashenko-Novokhatny AI, Zanina VV, Purish LM. [Microbial corrosion of underground struc-

tures]. Kyiv: Scientific opinion. 2005; 258 P. Russian.
19. Sukharevich VI, Kuzikova IL, Medvedeva IG, Gridneva YA. [Growth of micromycetes and synthesis of pigments on media containing fungicides and inhibitors of pigmentation]. Mycology and phytopathology. 2000; 34(3):43–7. Russian.
20. Kopteva ZhP, Zanina VV, Boretskaya MA, Yumyna UM, Kopteva AE, Kozlova IA. [Monosaccharide and fatty acid composition of the ekzopolymer complex of bacteria-destructors of the protective coating of the gas pipeline]. Microbiol Z. 2012; 74 (2):22–8. Russian.
21. Vortman MYa, Abdulina DR, Kopteva ZhP, Kopteva AE, Lemeshko VM, Shevchenko VV. [Antimicrobial activity and ability to biodegrade guanidine-containing polyethylene oxide hydrogel]. Physico-chemical mechanics of materials. Special issue. 2020; 13:33–7. Ukrainian.

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